

Claims

1. A mutant of the soluble form of EC 1.1.99.17 also known as PQQ-dependent soluble glucose dehydrogenase (s-GDH) said mutant characterized in that it has an at least two-fold increased substrate specificity for glucose, as compared to at least one other selected sugar substrate.
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2. The mutant according to claim 1 further characterized in that said selected sugar is selected from the group consisting of maltose and galactose.
3. The mutant according to claim 1 further characterized in that said selected sugar is maltose.
- 10 4. The mutant of PQQ-dependent s-GDH according to Claim 1 further characterized in that said substrate specificity for glucose is improved at least 3-fold.
5. The mutant of PQQ-dependent s-GDH according to Claim 1 further characterized in that said substrate specificity for glucose is improved at least 5-fold.
6. A mutant of the soluble form of EC 1.1.99.17 also known as PQQ-dependent soluble glucose dehydrogenase (s-GDH) said mutant characterized in that
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- a) the substrate specific reactivity towards glucose is essentially comparable to that of the wild-type enzyme, and
- b) the substrate specific reactivity towards maltose is 30% or less as compared to the wild-type enzyme.
- 20 7. The mutant according to claim 6 further characterized in that said substrate specific reactivity towards maltose is 20% or less as compared to the wild-type enzyme.
8. The mutant of a PQQ-dependent s-GDH according to claim 6 further characterized in that the wild-type s-GDH is isolated from a strain of the *Acinetobacter* species group consisting of *A. calcoaceticus* and *A. baumannii*.

9. A mutant protein of PQQ-dependent s-GDH according to claim 6 comprising at least one amino acid residue substitution at an amino acid position selected from the group comprising positions 348 and 428 of the corresponding s-GDH wild-type sequence known from *A. calcoaceticus*.
- 5 10. The mutant protein of claim 9 further characterized in that the amino acid residue threonine at position 348 is substituted with an amino acid residue selected from the group consisting of alanine, glycine and serine.
11. The mutant of claim 10 further characterized in that at least one of the following amino acid residues 16, 116, 120, 127, 169, 171, 177, 227, 255, 277, 299, 317, 355 and 438 is 10 also substituted.
12. The mutant of claim 9 further characterized in that asparagine at position 428 is substituted with an amino acid residue selected from the group consisting of leucine, proline and valine.
13. A mutant protein of PQQ-dependent s-GDH according to claim 1 comprising at least 15 two amino acid residue substitutions, said substituted amino acid positions being selected from the group consisting of positions 16, 22, 76, 116, 120, 127, 143, 168, 169, 171, 177, 227, 231, 255, 277, 295, 299, 308, 317, 348, 355, 422, 428 and 438. of the corresponding mature *A. calcoaceticus* soluble PQQ-dependent s-GDH, characterized in that at least one of the amino acid residues, T348 or N428 is replaced.
- 20 14. The mutant protein of claim 13, further characterized in that at least two of the amino acids in positions 76, 348 and 428 are substituted.
15. The mutant protein of claim 13 comprising substitutions of the amino acid residues at positions 348 and 428.
16. A mutant protein of PQQ-dependent s-GDH comprising the amino acid sequence of 25 WPXaaVAPS (SEQ ID NO: 1), wherein said Xaa residue is an amino acid residue other than threonine.

17. The mutant protein of claim 16 further characterized in that said Xaa residue is glycine.
18. A mutant protein of PQQ-dependent s-GDH comprising the amino acid sequence of TAGXaaVQK (SEQ ID NO: 2), wherein said Xaa residue is an amino acid residue other than asparagine.
- 5 19. The mutant protein of claim 18 further characterized in that said Xaa residue is proline.
20. A mutant protein of PQQ-dependent s-GDH comprising the amino acid sequence of ADGXaaNGL (SEQ ID NO: 3), wherein said Xaa residue is an amino acid residue other than glutamine.
- 10 21. The mutant of claim 20 further characterized in that said Xaa residue is selected from the group consisting of aspartic acid, glutamic acid, methionine, proline, serine, alanine or glycine.
22. An isolated polynucleotide encoding the s-GDH mutant protein according to any of claims 9 to 21.
- 15 23. An expression vector comprising an isolated polynucleotide as defined in claim 22 operably linked to a promoter sequence capable of promoting the expression of said polynucleotide in a host cell.
24. A host cell comprising the expression vector of claim 23.
25. A process for producing s-GDH variants comprising culturing the host cell of claim 24 under conditions suitable for production of the enzyme variants.
- 20 26. An expression vector comprising an isolated polynucleotide as defined in claim 22 operably linked to a promoter sequence capable of promoting its expression in a cell-free peptide synthesis system.
27. A process for producing s-GDH variants with the construct of claim 26 in a cell-free peptide synthesis system under conditions suitable for production of the said enzyme variants.

28. An improved method of detecting, determining or measuring glucose in a sample using a s-GDH mutant according to any of the proceeding claims, said improvement comprising a more specific detection of glucose.
29. The method of claim 28 further characterized in that said detection, determination or measurement of glucose is performed using a sensor or test strip device.
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30. A device for the detection or measurement of glucose in a sample comprising a s-GDH mutant according to any of claims 1- 29 and other reagents required for said measurement.